IN THE SPECIFICATION:

Please insert the following on page 1, between lines 3-4:

Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 2, 2010, is named ELLIS000.txt and is 10,942 bytes in size.

Please delete the paragraph on page 3, lines 11-24 and replace it with the following paragraph:

The Japanese patents JP02076598 and JP04169195 refer to anti-ED-B antibodies. It is not clear from these documents if monoclonal anti ED-B antibodies are described. Moreover, it seems impossible that a single antibody (such as the antibody described in JP02076598) has "an antigen determinant in amino acid sequence of formulae (1), (2) or (3):

- (1) EGIPIFEDFVDSSVGY **(SEQ ID NO: 22)**
- (2) YTVTGLEPGIDYDIS **(SEQ ID NO: 23)**
- (3) NGGESAPTTLTQQT **(SEQ ID NO: 24)**

on the basis of the following evidence:

i) A monoclonal antibody should recognize a well-defined epitope.

ii) The three-dimensional structure of the ED-B domain of fibronectin has been determined by NMR spectroscopy. Segments (1), (2) and (3) lie on opposite faces of the ED-B structure, and cannot be bound simultaneously by one monoclonal antibody.

Please delete page 11, line 1 to page 12, line 10, and replace with the following:

The dose of radioactivity delivered per gram of tumour is proportional to the area under the curve (AUC) of the % ID/g (tumour), plotted versus time. Analogously, the dose of radioactivity delivered to blood is proportional to the area under the curve (AUC) of the % ID/g (blood), plotted versus time. During the first 24 hours after intravenous injection the ratio of AUC

(tumour)/AUC (blood) is equal to 3.6 (Figure 12~~13~~). This ratio may somewhat increase when the AUCs are measured for longer time periods, but therapeutic ratios greater than 6-7 are rarely achieved. Let us now consider the situation in which a short-range-acting toxic species (e.g., a photosensitiser or an alpha-particle emitting radionuclide) is delivered to the tumoural blood vessels, and is therefore not homogenously distributed in the tumour mass. The diffusive toxic species (e.g., singlet oxygen or an alpha particle) will hardly penetrate more than one layer of cells, i.e. will hit only the endothelial cells lining the tumour blood vessel wall. Since new-forming blood vessels in most tumours (including the F9 teratocarcinoma) constitute less than 2% of the total tumour mass, the relevant parameter for predicting therapeutic benefit will be the ratio AUC(vessel)/AUC(blood).

As we can see in Figure 13~~14~~, L19 accumulates only around new blood vessels. This observation has also been confirmed by microautoradiographic analysis in tumours (Tarli et al. 1999, Blood, 94, 192-198). Since the radioactivity delivered to tumours is concentrated around neo-vascular structures, and assuming that tumoural vasculature accounts for 2% or the tumour weight, we can calculate that AUC (vessel) = (100/2) x AUC (tumour) = 50 x AUC (tumour). In other words, the selectivity of the treatment is expected to be proportional to the ratio AUC (vessel) / AUC (blood), which is > 150:1 over a period of 24 hours in our experimental system (see also Figure 14~~15~~ for explanation).

Let us consider astatine-211 as a particularly interesting alpha-particle emitting radionuclide for biomedical application. The half-life of its radioactive decay is considerably longer than the ones of bismuth-212 and bismuth-213 (7.2 hrs, compared to 1.0 hr and 45 min., respectively) (Larsen and Bruland 1998, Brit. J. Cancer 77, 1115-1122). Astatine is the heaviest halogen and no stable isotopes of this element exist. Since it is directly below iodine in the periodic table, it might be expected that the two halogens would possess similar chemical properties. However, attempts to label proteins with ^{211}At by direct electrophilic astatination at the level of the protein aminoacid did not give successful results because of rapid loss of label following in vivo administration (Vaughan et al. 1978, Int. J. Nucl. Med. 5, 229-230). To circumvent this problem, L19 can be conjugated with ^{211}At with significant nuclide incorporation and no loss of antibody specificity by the two-step labeling method published by Garg (Garg et al. 1989, Appl. Radiat. Isot. 40, 485-490). It involves the use of a bifunctional chemical compound, N-succinimidyl-3-

(trimethylstannyl)-benzoate (m-MeATE; ATE, <<alkyltin ester>>) (Zalutsky et al. 1989, Proc. Natl. Acad. Sci; USA 86, 7149-7153), which we synthesized following the protocol published by Garg (see Figure 15~~16~~) as reported in Example 7.

Please delete page 14, lines 2-27, and replace with the following:

~~Fig. 6 shows amino acid sequence of L19 (VH, SEQ ID NO. 19);~~

Fig. 6~~7~~ shows rabbit eyes with implanted pellet;

Fig. 7~~8~~ shows immunohistochemistry of rabbit cornea sections.

Fig. 8~~9~~ shows the immunohistochemistry of sections of ocular structures of rabbits (cornea, iris and conjunctiva) using a red alkaline phoshatase substrate and hematoxylin.

Fig. 9~~10~~ shows the localisation of fluorescently-labelled antibodies in ocular neovasculature.

Fig. 10~~11~~ shows the macroscopic appearance of the eyes of rabbits injected with proteins coupled to photosensitizers, before and after irradiation.

Fig. 11~~12~~ shows the microscopic analysis of sections of ocular structures of rabbits injected with proteins coupled to photosensitizers and irradiated with red light.

Fig. 12~~13~~ shows schematic diagrams in which the % of injected dose delivered per gram, respectively of blood and tumour, are plotted versus time.

Fig. 13~~14~~ shows the accumulation of L19 around new vessels.

Fig. 14~~15~~ shows schematic diagrams reporting the relevant % of injected doses per gram, in the case of the L19 antibody coupled to an .alpha.- and .beta.-emitter respectively, plotted versus time.

Fig. 15~~16~~ shows the labelling method according to the Garg protocol modified as described hereinafter.

Figs. 16a and 16b~~17a and 17b~~ report the 1H-NMR spectrum of 3-(trimethylstannyl)-benzoic acid.

Figs. 17a and 17b~~18a and 18b~~ report the 1 H-NMR spectrum of N-succinimidyl-3-(trimethylstannyl)-benzoate

Fig. 18~~19~~ shows the EI-MS of N-succinimidyl-3-(trimethylstannyl)-benzoate.

Please delete page 16, line 10 – page 19, line 3, and replace with the following:

~~FIG. 6 shows the amino acid sequence of antibody L19 comprising the heavy chain (VH), the linker and the light chain (VL).~~

Fig. 6~~7~~ shows rabbit eyes with implanted polymer pellets soaked with angiogenic substances.

Fig. 7~~8~~ shows immunohistochemistry of sections of rabbit cornea with new-forming blood vessels, stained with the L19 antibody.

Fig. 8~~9~~ shows immunohistochemical studies of ocular structures using the L19 antibody. A specific red staining is observed around neovascular structures in the cornea (Figure 8a~~9a~~), but not around blood vessels in the iris (Figure 8b~~9b~~) and in the conjunctiva (Figure 8c~~9c~~). Small arrows: corneal epithelium. Relevant blood vessels are indicated with large arrows. Scale bars: 50 µm

Fig. 9~~10~~ shows immunophotodetection of fluorescently labelled antibodies targeting ocular angiogenesis. A strongly fluorescent corneal neovascularisation (indicated by an arrow) is observed in rabbits injected with the antibody conjugate L19-Cy5 (Figure 9a~~10a~~), specific for the ED-B domain of FN, but not with the antibody HyHEL-10-Cy5 (Figure 9b~~10b~~). Immunofluorescence microscopy on cornea sections confirmed that L19-Cy5 (Figure 9c~~10c~~), but not HyHEL-10-Cy5 (Figure 9d~~10d~~) localises around neovascular structures in the cornea. Images (Figure 9a,b~~10a,b~~) were acquired 8 h after antibody injection; (Figure 9c,d~~10c,d~~) were obtained using cornea sections isolated from rabbits 24 h after antibody injection. P, pellet.

Fig. 10~~11~~ shows macroscopic images of eyes of rabbits treated with photosensitiser conjugates. Eye of rabbit injected with L19-PS before (Figure 10a~~11a~~) and 16 h after irradiation with red light (Figure 10b~~11b~~). The arrow indicates coagulated neovasculature, which is confirmed as a hypofluorescent area in the Cy5 fluoroangiogram of panel (Figure 10c~~11c~~) 16 h after irradiation. Note that no coagulation is observed in other vascular structures, for example in the dilated conjuctival vessels. For comparison, a Cy5 fluoroangiogram with hyperfluorescence of leaky vessels, and the corresponding colour photograph of untreated rabbit eye are shown in (Figure 10d~~11d~~) and (Figure 10h~~11h~~). Pictures (Figures 10e, 10f, 10g~~11e, 11f, 11g~~) are analogous to (Figures 10a, 10b, 10c~~11a, 11b, 11c~~), but correspond to a rabbit injected with ovalbumin-PS and irradiated with red light. No coagulation can be observed, and the angiogram reveals hyperfluorescence of leaky vessels. The eyes of rabbits with early-stage angiogenesis and injected with L19-PS are shown in (Figures 10i, 10l~~11i, 11l~~). Images before (Figure 10i~~11i~~) and 16 h after

irradiation with red light (Figure 10j~~11j~~) reveal extensive and selective light-induced intravascular coagulation (arrow). Vessel occlusion (arrow) is particularly evident in the irradiated eye (Figure 10l~~11l~~) of a rabbit immediately after euthanasia, but cannot be detected in the non-irradiated eye (Figure 10k~~11k~~) of the same rabbit. P, pellet. Arrowheads indicate the corneo-scleral junction (limbus). In all figures, dilated pre-existing conjunctival vessels are visible above the limbus, whereas growth of corneal neovascularisation can be observed from the limbus towards the pellet (P).

Fig. 11~~12~~ shows microscopic analysis of selective blood vessel occlusion. H/E sections of corneas (a,e,b,f: non-fixed; i,j: paraformaldehyde fixed) of rabbits injected with ovalbumin-PS (Figures 11a, 11e, 11i~~12a, 13e, 12i~~) or L19-PS (Figures 11b, 11f, 11j~~12b, 12f, 12j~~) and irradiated. Large arrows indicate representative non damaged (Figures 11e, 11i~~12e, 12i~~) or completely occluded (Figures 11f, 11j~~12f, 12j~~) blood vessels. In contrast to the selective occlusion of corneal neovasculature and restricted perivascular damage (eosinophilia) mediated by L19-PS after irradiation (Figures 11b, 11f, 11j~~12b, 12f, 12j~~), vessels in the conjunctiva (Figure 11k~~12k~~) and iris (Figure 11l~~12l~~) do not show sign of damage in the same rabbit. Fluorescent TUNEL assay indicates the different number of apoptotic cells in sections of irradiated rabbits injected with L19-PS (11c, 11g~~12e, 12g~~) or with ovalbumin-PS (11d, 11h~~12d, 12h~~). Large arrows indicate some relevant vascular structures. Small arrows indicate corneal epithelium. Scale bars: 100 µm (11a-11d~~12a-12d~~) and 25 µm (11e-11l~~12e-12l~~)

Fig. 12~~13~~ shows the area under the curve (AUC) of the radioactivity delivered by scFv(L19) to the blood and to the tumour during the first 24 hours after intravenous injection. In this experimental study performed in mice bearing the F9 teratocarcinoma, the AUC of the injected dose of radiolabelled antibody delivered per gram of tumour is 3.6-fold higher than the dose delivered per gram of blood. This ratio increases when the AUC is measured for longer time periods.

Fig. 13~~14~~ shows microautoradiographic analysis of an F9 teratocarcinoma dissected from a nude mouse, after injection of radiolabelled scFv(L19). The pictures show that scFv(L19) accumulates around vascular structure but not in the surrounding normal mouse tissue.

Fig. 14~~15~~ illustrates a schematic diagram of the radiommunotherapy performed with the anti-angiogenesis scFv(L19) coupled with .beta.- or .alpha.-emitting radionuclide. Because new-

forming blood vessels in F9 teratocarcinoma constitute 0.5-5% of the total tumour mass, the radioactivity delivered to vascular structures is significantly higher (70-700-fold) than the one delivered to normal tissue and to blood. Coupling scFv(L19) to a beta emitter (e.g., Yttrium-90), the majority of the targeted tumoural area is irradiated, since these .beta.-particles have a range in tissue of several millimiters. On the other hand, coupling scFv(L19) to an alpha emitter (e.g. Astatine-211 or Bismuth-212 or Bismuth-213), the radiation is deposited only around the targeted tumoural blood vessels (penetration: few dozens of micrometers). In this case, the relevant parameters for therapeutic efficacy is the vessel:blood ratio of the percent injected dose of radioactivity per gram of tissue, rather than the tumour:blood ratio (which is the relevant parameter for beta emitting nuclides).

Fig. 15~~16~~ shows the procedure for labelling scFv(L19) with Iodine-125 and Astatine-211 using the N-succinimidyl 3-(trimethylstannyl)benzoate (m-MeATE) synthesized from m-bromobenzoic acid.

Fig. 16a~~17A~~ shows the ^{1}H-NMR spectrum of 3-(trimethylstannyl)benzoic acid in $CDCl_3$. The chemical shifts (ppm) are indicated in the X-axis.

Fig. 16b~~17B~~ shows the chemical shift in ppm of the protons in the aromatic moiety located at low magnetic field of the NMR spectrum.

Fig. 17a~~18A~~ shows the ^{1}H-NMR spectrum of m-MeATE in $CDCl_3$. The relative values of peak intensity are reported between the X-axis reporting the chemical shift (ppm) and the spectrum baseline.

Fig. 17b~~18B~~ shows an enlarged portion of the ^{1}H-NMR spectrum of m-MeATE.

Please delete page 19, lines 1 - 3, and replace with the following:

Fig. 18~~19~~ shows the electron ionization mass spectrum (EI-MS) of m-MeATE (molecular weight 383). The mass to charge value (m/e) of 368 represents the mass of the molecular ion (M^+)-15 (CH_3).

Please replace the paragraphs beginning at page 26, line 27 and ending at page 27, line 29, with the following:

We investigated whether B-FN is a specific marker of ocular angiogenesis and whether antibodies recognising B-FN could selectively target ocular neovascular structures in vivo upon systemic administration. To this aim we stimulated angiogenesis in the rabbit cornea, which allows the direct observation of new-blood vessels, by surgically implanting pellets containing vascular endothelial growth factor or a phorbol ester (Fig. 67). Sucralfate (kind gift of Merck, Darmstadt, Germany)/hydron pellets containing either 800 ng vascular endothelial growth factor (Sigma) or 400 ng phorbol 12-myristate 13-acetate (<<PMA>>; Sigma) were implanted in the cornea of New Zealand White female rabbits as described [D'Amato, R. J., et al., Proc. Natl. Acad. Sci. USA 91, 4082-4085 (1994)]. Angiogenesis was induced by both factors. Rabbits were monitored daily. With both inducers newly formed blood vessels were strongly ED-B-positive in immunohistochemistry. For all further experiments, PMA pellets were used.
Immunohistochemical studies showed that L19 strongly stains the neovasculature induced in the rabbit cornea (Fig. 78; 89a), but not pre-existing blood vessels of the eye (Fig. 89b, c) and of other tissues (data not shown). Immunohistochemistry was performed as described [Carnemolla, B. et al., Int. J. Cancer 68, 397-405 (1996)].

EXAMPLE 5

The Human Antibody Fragment L19, Binding to the ED-B with Sub-Nanomolar Affinity, Targets Ocular Angiogenesis *In Vivo*

Using the rabbit cornea model of angiogenesis described in the previous example, and an immunophotodetection methodology [Neri, D. et al., Nature Biotechnol. 15, 1271-1275 (1997)], we demonstrated that L19, chemically coupled to the red fluorophore Cy5, but not the antibody fragment (HyHEL-10)-Cy5 directed against an irrelevant antigen (Fig. 910a,b), selectively targets ocular angiogenesis upon intravenous injection. Fluorescent staining of growing ocular vessels was clearly detectable with L19 immediately after injection, and persisted for at least two days analogous to previous observations with tumour angiogenesis. Subsequent ex vivo immunofluorescent microscopic analysis on cornea sections confirmed the localisation of L19, but not of HyHEL-10, around vascular structures (Fig. 910c,d). The demonstration of the antibody-based selective targeting of ocular neovascularisation, together with the reactivity of anti-B-FN antibodies in different species, warrants future clinical investigations.

Immunofluorescence imaging could be useful for the early detection of ocular angiogenesis in risk patients, before lesions become manifest in fluoroangiography.

Please replace the paragraphs beginning at page 29, line 9 and ending at page 30, line 5, with the following:

To test whether selective vessel ablation could be achieved by virtue of the antibody-mediated targeting, we injected rabbits with the L19 antibody fragment or an irrelevant protein that does not localise in newly formed blood vessels (ovalbumin) coupled to the photosensitiser tin (IV) chlorin e_6 (hereafter named <<PS>>). The eyes of injected animals were irradiated with red light (light dose = 78 J/cm^2). Representative results are depicted in Fig. 10~~11~~. A striking macroscopic difference was observed 16 h after irradiation in rabbits treated with L19-PS (Fig. 10~~11~~a,b), with coagulation of the corneal neovasculature but not of vessels in the conjunctiva or in other ocular structures. Fluoroangiography with the indocyanine fluorophore Cy5 (Fig. 10~~11~~c) confirmed vessel occlusion as a characteristic hypofluorescent area. On the contrary, hyperfluorescent areas were observed in the leaky neovasculature of non-irradiated eyes (Fig. 10~~11~~d,h). No macroscopic alteration was detectable in the irradiated vessels of rabbits treated with ovalbumin-PS (Fig. 10~~11~~e-g), either ophthalmoscopically or by Cy5 fluoroangiography. The effect of irradiation of the targeted L19-PS conjugate at early stages of corneal angiogenesis are shown in Fig 10~~11~~i-l. Selectively coagulated blood vessels were macroscopically visible in live animals (Fig. 10~~11~~i,j) and even more evident in animals immediately after euthanasia (Fig. 10~~11~~k,l).
Photodynamic damage was further investigated using microscopic techniques. After irradiation, vessel occlusion could be detected by standard hematoxylin/eosin (H/E) staining techniques in both non-fixed and paraformaldehyde-fixed cornea sections of animals treated with L19-PS (Fig. 10~~11~~b,f,j), but not of those treated with ovalbumin-PS (Fig. 10~~11~~a,e,i). Apoptosis in the portion of the cornea targeted by the photosensitiser conjugate was clearly visible in the fluorescent TUNEL assay (Fig. 10~~11~~c,g), but hardly detectable ink negative controls (Fig. 10~~11~~d,h). A higher magnification view showed apoptosis of endothelial cells in vascular structures (Fig. 10~~11~~g). No damage to blood vessels of the iris, sclera and conjunctiva of treated animals could be observed either by TUNEL assay (not shown) or by H/E staining (Fig. 10~~11~~k,l).

Paragraph beginning at page 31, line 23 and ending at page 32, line 9, replace with the following:

Conjugation of Astatine-211 to Human Antibody Fragment L19 Through m-MeATE
A solution of m-bromobenzoic acid (1 g, 5 mmol) in 25 ml of dry tetrahydrofuran (THF) is placed in a 250 ml, two-necked round bottom flask, maintained under nitrogen atmosphere and cooled to -75 °C using (ether:dry ice) bath. To the above, 6.25 ml of n-buthyl lithium (1.6M solution in hexane) is added slowly over 25 min. The dilithio anion thus generated is stirred for an additional 10 min. Still under a nitrogen atmosphere, trimethylstannyl chloride (1.09 mg, 5.47 mmol) dissolved in 10 ml dry THF is added over 20 min to the reaction mixture. The cooling bath is removed and the reaction is slowly brought to room temperature (RT) and stirring is kept for 1 hour. The reaction is quenched by addition of water (10 ml) and extracted three times with 100 ml of diethyl ether. The organic phase is washed with 5% $NaHCO_3$ (2 x 25 ml) and water (2 x 20 ml). After drying over $MgSO_4$, it is concentrated on a rotary evaporator. Thin layer chromatography (TLC) is performed on analytical, pre-coated silica gel plastic plates using the following mobile phase: Hexane:ethylacetate(EtOAc):acetic acid (AcOH) (70:29.7:0.3). 3-(trimethylstannyl) benzoate (Rf=0.46) is purified by gravimetric chromatography using a silica gel 60 column (40-63μ, 200 x 3 mm) eluted with the same phase above reported. We obtain 100 mg (0.35 mmol) of product, in 7% yield as an oil. The assigned structure (^{1}H-NMR) is reported in Fig. 16~~17~~a,b.

Page 32, lines 10-19, replace with the following:

To the stannyl ester resuspended in THF (10 ml), N-hydroxy-succinimide (48.34 mg, 0.42 mmol) and diciclohexylcarbodiimide (86.66 mg, 0.42 mmol) are added and stirred overnight at room temperature. Precipitated dicyclohexylurea is filtered off and the solvent is evaporated on a rotary evaporator to give an oil. The desired m-MeATE (Rf=0.34) is isolated by gravimetric chromatography with Hexane:EtOAc (2:1) using a silica gel 60 column. After evaporation of the solvent we obtain 92.3 mg (0.241 mmol) of m-MeATE in 68.8% relative yield. The assigned

structure is confirmed by ^{1}H-NMR and Mass Spectroscopy (Figs. 17~~18~~a,b and 18~~19~~). This bifunctional compound is used to couple scFv(L19) to the alpha-emitting radionuclide Astatine-211.